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Interactions between the Tetrasodium Salts of EDTA and 1-Hydroxyethane 1,1-Diphosphonic Acid with Sodium Hypochlorite Irrigants

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Interactions between the Tetrasodium Salts of EDTA and 1-Hydroxyethane 1,1-Diphosphonic Acid with Sodium Hypochlorite Irrigants

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Abstract

Introduction: A clinically useful all-in-one endodontic irrigant with combined proteolytic and decalcifying properties is still elusive. In this study, the chemical effects of dissolving the tetrasodium salts of 1-hydroxyethane 1,1-diphosphonic acid (Na_4HEDP) or Na_4EDTA directly in sodium hypochlorite (NaOCl) irrigants in polypropylene syringes were assessed during the course of 1 hour. **Methods:** The solubility of the salts in water was determined. Their compatibility with 1% and 5% NaOCl was measured by iodometric titration and in a calcium complexation experiment by using a Ca^{2+}-selective electrode. **Results:** The salts dissolved within 1 minute. The dissolution maximum of Na_4HEDP in water (wt/total wt) was 44.6% ± 1.6%. The corresponding dissolution maximum of Na_4EDTA was 38.2% ± 0.8%. Na_4HEDP at 18% in 5% NaOCl caused a mere loss of 16% of the initially available chlorine during 1 hour. In contrast, a corresponding mixture between NaOCl and the Na_4EDTA salt caused 95% reduction in available chlorine after 1 minute. Mixtures of 3% Na_4EDTA with 1% NaOCl were more stable, but only for 30 minutes. Na_4HEDP lost 24% of its calcium complexation capacity after 60 minutes. The corresponding loss for Na_4EDTA was 34%. **Conclusions:** The compatibility and solubility of particulate Na_4HEDP with/in NaOCl solutions are such that these components can be mixed and used for up to 1 hour. In contrast, short-term compatibility of the Na_4EDTA salt with NaOCl solutions was considerably lower, decreasing at higher concentrations of either compound. Especially for Na_4HEDP but also for Na_4EDTA, the NaOCl had little effect on calcium complexation. *(J Endod 2017;43:657–661)*

Key Words

EDTA, etidronate, HEBP, HEDP, NaOCl, sodium hypochlorite

Significance

The results of the current study suggest a possibility that a combination of Na_4HEDP salt directly dissolved in a NaOCl solution may be used when mixed immediately before treatment as a sole endodontic irrigant for at least 1 hour.

The term *chemomechanical root canal preparation* relates to the clinical concept of cleaning infected and/or inflamed root canal systems by means of instrumentation and irrigation with a chemically active substance. The goal is to clean the canal system and condition the canal wall for a subsequent filling procedure. Current approaches aim to remove the inflamed or necrotic soft tissue, smear layer, and biofilm, if present. Subsequently, the canal wall can be conditioned to improve the attachment of alloplastic filling materials (1) or to release growth factors to engineer a pulp-like tissue (2).

Alkaline sodium hypochlorite (NaOCl) solutions are the most widely used endodontic irrigants (3, 4). These NaOCl solutions are relatively cheap and readily available from various commercial sources (5). Furthermore, NaOCl solutions feature unique soft tissue and biofilm matrix dissolution properties (6, 7). However, irrigation with NaOCl solutions has no effect on the inorganic aspects of the smear layer (8) and does not prevent the accumulation of inorganic debris during instrumentation (9). Consequently, irrigation protocols using NaOCl in an alternating mode with an aqueous solution containing a decalcifying agent such as EDTA or citric acid have been proposed (10). However, these protocols are not necessarily user-friendly. Because of the chemical reactivity of OCl^- (hypochlorite) with most decalcifying agents, especially those in acidic form, combined use is not possible (11, 12). The search for hypochlorite-compatible decalcifying agents or chelators in endodontic research has yielded 2 candidates thus far, tetrasodium 1-hydroxyethane 1,1-diphosphonic acid (HEDP) (Na_4HEDP) (12, 13) and tetrasodium EDTA (Na_4EDTA) (14, 15). Both HEDP (or etidronate) and EDTA are available as disodium and tetrasodium salts, depending on the desired pH. These biocompatible chelators are commonly used in the cosmetic, food and dish washing, and the laundry detergent industry (16). HEDP has a higher calcium binding capacity per gram chelator (16), whereas EDTA has a stronger affinity to calcium, ie, a higher dissociation constant of the complex (17). Consequently, EDTA has a higher demineralizing effect than HEDP and can erode dentin (18). EDTA solutions of a concentration lower than the usual 17% have thus been proposed. It has been shown that 3% EDTA can remove smear layer as effectively as 24% EDTA (19). It would appear that the reduction of the available chlorine that occurs in direct mixtures with Na_4HEDP is less than that in counterparts containing Na_4EDTA (12, 15). However, a direct

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comparison is elusive. Furthermore, it is not known how the calcium binding capacity (complexation) of these 2 chelators is affected by the presence of NaOCl in aqueous mixtures.

It is known from previous research that no chelator investigated thus far has true long-term compatibility with NaOCl (12). Consequently, it was the goal of the present investigation to study short-term chemical interactions between the tetrasodium salts of HEDP and EDTA with NaOCl irrigants at different concentrations recommended by previous research. To simulate a clinical scenario, mixtures were made directly in 10-mL polypropylene irrigation syringes.

Materials and Methods

Particles and Solutions

The tetrasodium salts of HEDP and EDTA were obtained from commercial sources (HEDP: Cublen K8514 GR, Zschimmer & Schwarz, Burgstädt, Germany, batch no. 92729; EDTA: Sigma-Aldrich, Steinheim, Germany, batch no. 058K0136). The salts were sieved through a stack of precision sieves with defined screen openings (Test Sieve; Retsch GmbH, Haan, Germany). Particles from the 300- to 500-μm fraction were used for all subsequent experiments.

The solutions used in these experiments were 5.0% and 1.0% NaOCl. They were diluted from a concentrated 14% stock solution by using pure reagent-grade water. The stock solution was kept at 5°C in an amber glass bottle. Diluted solutions were prepared freshly before each experiment and warmed to ambient temperature. Available chlorine was determined and verified by using iodometric titration (20).

Dissolution Maximum

The particles were dissolved in 25 mL deionized water at room temperature. The powders were incrementally added under constant stirring until the particles stopped to dissolve. This procedure was repeated twice per chelator salt under investigation (triplicate experiments). Subsequently, the solutions were filtered by using a 0.22-μm syringe filter (TPP Techno Plastic Products AG, Trasadingen, Switzerland). To determine the weight of the dissolved salts per total weight of solution, 5 mL of the filtered solutions was pipetted into a pre-weighed glass vial. Weights were determined by using a precision balance (Mettler AT261 DeltaRange; Mettler Toledo Int Inc, Columbus, OH). Subsequently, the probes were desiccated in a drying oven (HORO type 038A; Dr. Ing. A. Hoffmann, Ostfildern, Germany) at 120°C until all the water had evaporated and a steady weight was achieved.

Effect of Chelator Salts on Available Chlorine

In this experiment, the chelator salts were added to and dissolved in pre-weighed amounts of 1% or 5% NaOCl. The mixtures were prepared directly in 10-mL polypropylene syringes (Omnifix REF4617100 V; B. Braun, Melsungen, Germany). The plungers were removed, and the syringe openings were closed by using paraffin film (Parafilm; Bemis, Neenah, WI). The NaOCl solution and the chelator salt were added, and the syringe hubs were closed by using Parafilm (Bemis). Experiments were performed in triplicates at the intervals and concentration measured, ie, 3 separate syringes were prepared each (total of 150 syringes for these experiments; Tables 1 and 2). Experiments were performed at ambient temperature. The triplicate samples per concentration/time were placed in a centrifuge tube rack and gently shaken by hand for 1 minute. By that time, the chelator salts had dissolved completely. Aliquots of the mixed solutions, approximately 0.5 g, were weighed in the precision balance. They were removed after 1, 10, 20, 30, and 60 minutes and assessed for their amount of available chlorine by using a titration apparatus (665 Dosimat; Deutsche Metrohm GmbH & Co KG, Filderstadt, Germany). A 0.1 mol/L sodium thiosulfate solution (Merck KGaA Darmstadt, Darmstadt, Germany; lot no. HC378976) was used as titrator. In addition, the temperature and the pH of the reaction mixtures were recorded. Temperature measurements were performed by using an electronic thermometer (Checktemp 1; Hanna Instruments, Leighton Buzzard, Great Britain) and pH measurements by using a calibrated microelectrode (827 pH lab; Deutsche Metrohm GmbH & Co KG).

Effect of Hypochlorite on Ca^{2+} Complexing Capacity

To study the effect of the interactions between the hypochlorite and the chelators under investigation, the salts were added to a 5% NaOCl solution. (From the available chlorine experiment), it was expected that there was more interaction in this solution than in the 1% counterpart.

TABLE 1. Available Chlorine (% of Theoretical Maximum), pH, and Temperature (°C) in 10 mL of 1.0% NaOCl Solution in Polypropylene Syringe after Addition of the Chelator Salt

Salt (wt/wt)	1 min	10 min	20 min	30 min	60 min
18% Na_4HEDP					
Available chlorine (%)	95 ± 2	92 ± 2	88 ± 2	83 ± 2	72 ± 3
pH range	11.6	11.5–11.7	11.4–11.6	11.3–11.5	11.1–11.2
Temperature (°C)	24 ± 0	24 ± 0	24 ± 0	23 ± 0	23 ± 0
9% Na_4HEDP					
Available chlorine (%)	99 ± 1	97 ± 0	95 ± 0	93 ± 1	87 ± 3
pH range	11.0–11.2	11.1–11.2	11.1	11.0–11.1	10.9–11.0
Temperature (°C)	22 ± 0	22 ± 0	22 ± 0	22 ± 0	22 ± 0
18% Na_4EDTA					
Available chlorine (%)	73 ± 1	10 ± 1	8 ± 0	7 ± 1	6 ± 2
pH range	11.2–11.5	9.8	9.7–9.8	9.7–9.8	9.7
Temperature (°C)	21 ± 1	25 ± 0	23 ± 0	22 ± 0	21 ± 1
3% Na_4EDTA					
Available chlorine (%)	95 ± 1	93 ± 1	90 ± 1	87 ± 1	17 ± 0
pH range	12.1–12.6	12.1–12.2	11.5–12.0	11.5–11.9	9.7–9.8
Temperature (°C)	20 ± 1	21 ± 0	21 ± 0	21 ± 0	23 ± 1
None (control)					
Available chlorine (%)	100 ± 1	99 ± 0	100 ± 1	100 ± 2	99 ± 1
pH range	11.7	11.6–11.8	11.7	11.7–11.8	11.6–11.8
Temperature (°C)	22 ± 1	22 ± 0	22 ± 0	22 ± 0	22 ± 0

Triplicate experiments, means and standard deviations except pH: total ranges.

TABLE 2. Available Chlorine (% of Theoretical Maximum), pH, and Temperature (°C) in 10 mL of 5.0% NaOCl Solution in Polypropylene Syringe after Addition of Chelator Salt

Salt (wt/wt)	1 min	10 min	20 min	30 min	60 min
18% Na_4HEDP					
Available chlorine (%)	100 ± 0	99 ± 4	95 ± 5	95 ± 5	84 ± 8
pH range	12.3–12.7	11.9–12.6	12.1–12.3	11.9–12.4	10.5–11.6
Temperature (°C)	23 ± 2	24 ± 2	24 ± 2	24 ± 1	25 ± 2
9% Na_4HEDP					
Available chlorine (%)	98 ± 1	98 ± 1	96 ± 1	94 ± 2	86 ± 2
pH range	12.3	11.9–12.3	11.5–12.1	10.8–11.9	9.9–10.4
Temperature (°C)	22 ± 1	22 ± 1	22 ± 1	23 ± 1	24 ± 1
18% Na_4EDTA					
Available chlorine (%)	5 ± 4	1 ± 0	1 ± 0	1 ± 0	1 ± 0
pH range	8.8–11.8	8.6–8.7	8.6–8.7	8.6–8.7	8.6–8.7
Temperature (°C)	38 ± 10	33 ± 2	28 ± 1	26 ± 0	22 ± 0
3% Na_4EDTA					
Available chlorine (%)	96 ± 1	5 ± 0	3 ± 0	3 ± 0	3 ± 0
pH range	10.3–10.9	7.0–7.5	6.9–7.0	6.9–7.0	6.9–7.0
Temperature (°C)	22 ± 1	49 ± 3	37 ± 1	30 ± 1	26 ± 1
None (control)					
Available chlorine (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
pH range	12.4–12.5	12.4–12.6	12.3–12.5	12.5–12.7	12.5–12.6
Temperature (°C)	22 ± 0	22 ± 0	22 ± 0	22 ± 0	22 ± 0

Triplicate experiments, means and standard deviations except pH: total ranges.

Again, mixtures were prepared in individual syringes in triplicates for each time point and combination as described above (36 syringes in total; Table 3). To stop the reaction, 0.6 g sodium thiosulfate was added to the mix immediately, after 30 or 60 minutes. To determine the Ca^{2+} complexing capacity of the 2 chelators in the presence of NaOCl, a calcium chloride standard solution ($CaCl_2$ * 2 H_2O, 18.35 g/5 L, EMSURE; Merck, Kenilworth, NJ) was used instead of the commonly used calcium carbonate standard. The concentration of the standard solution (1000 ppm Ca^{2+}, pH 6.1) was verified by using an atomic absorption spectrometer (contrAA 300; Analytik Jena AG, Jena, Germany) and compared with that of a commercially available 1000 ppm Ca^{2+} standard solution (CertiPUR calcium carbonate; Merck, no. 1.19778.0100). A pilot study revealed that the pH effects of NaOCl gave rise to false readings when using a calcium carbonate standard solution (pH 1), which needs to be acidic (data not shown). Thus, the experiments had to be performed in a high-pH environment. The $CaCl_2$ solution was buffered at pH 10 by using a commercially available boric acid/potassium chloride/sodium hydroxide buffer (2 mL per 100 mL standard solution, EMSURE; Merck). For the experiment the 1000 ppm Ca^{2+} standard solution was diluted to 100 ppm. The potential of 100 mL of this solution was determined by using a calcium-selective electrode (ORION 93 series 9300 BN; Thermo Fisher Scientific, Waltham, MA). Subsequently, 0.1-mL increments of the reaction mixtures were added to 100 mL of the 100 ppm Ca^{2+} standard solution. Control measurements were performed by using corresponding amounts of 6% sodium thiosulfate in 5% NaOCl or deionized water. Measurements were performed against an exponential 5-point standard curve, and corresponding control values were subtracted.

TABLE 3. Ca^{2+} That Was Chelated (% Reduction) When 0.2 mL of Mixtures of Tetrasodium Salts of HEDP or EDTA with Deionized Water or Sodium Hypochlorite Solutions Preincubated in 10-mL Polypropylene Syringes for Different Time Intervals Were Added to 100 mL of 100 ppm Calcium Chloride Standard Solution

Mixture (wt/wt)	Immediately	30 min	60 min
18% HEDP in water (%)	82 ± 1	83 ± 1	82 ± 2
18% HEDP in 5% NaOCl (%)	75 ± 1	68 ± 2	62 ± 3
18% EDTA in water (%)	39 ± 1	40 ± 3	44 ± 1
18% EDTA in 5% NaOCl (%)	34 ± 3	30 ± 4	29 ± 3

Triplicate experiments, means and standard deviations.

Data Presentation

All the percentage (%) values reported relate to mass, ie, weight of chemicals per total weight of the resulting solution. The data presented are based on triplicate experiments. Mean values and standard deviations are shown. Because these data represent results from chemical experiments with little variance, only descriptive statistics were applied.

Results

Both the Na_4HEDP and Na_4EDTA salts dissolved in the NaOCl solutions within 1 minute. The dissolution maximum of Na_4HEDP in water (wt/total wt) was 44.6% ± 1.6%. The corresponding dissolution maximum of Na_4EDTA was 38.2% ± 0.8%.

Both chelator salts showed some negative effects on free available chlorine when dissolved in NaOCl solutions (Tables 1 and 2). When Na_4HEDP powder was mixed with 1% or 5% NaOCl, between 72% and 87% of the initially available chlorine remained in solution after 60 minutes (Tables 1 and 2). The higher concentration of HEDP (18%) caused a slightly higher loss of available chlorine than the lower concentration (9%), whereas the concentration of the NaOCl solution had little impact (Fig. 1). In contrast, Na_4EDTA showed some strong and dose-dependent effects, especially in 5% NaOCl (Table 2). When 18% Na_4EDTA was present in 5% NaOCl, the reaction was immediate (Fig. 1), with a concurrent drop in pH and rise in temperature (Table 2). In the presence of 3% Na_4EDTA, 5% NaOCl reacted in the first 10 minutes, whereas the 1% solution retained 87% of the available chlorine in the first 30 minutes (Table 1).

The tetrasodium salt of HEDP in 5% NaOCl initially lost 9% of its calcium chelating effect compared with a pure solution in water (Table 3) and 24% after 60 minutes. These losses correlated to the time course of the interaction with the available chlorine (Table 2). The corresponding immediate loss for Na_4EDTA was 13%, reaching 34% after 60 minutes. Consequently, despite its apparent interaction with the hypochlorite (OCl^-), Na_4EDTA retained its ability to chelate Ca^{2+} during the course of this experiment.

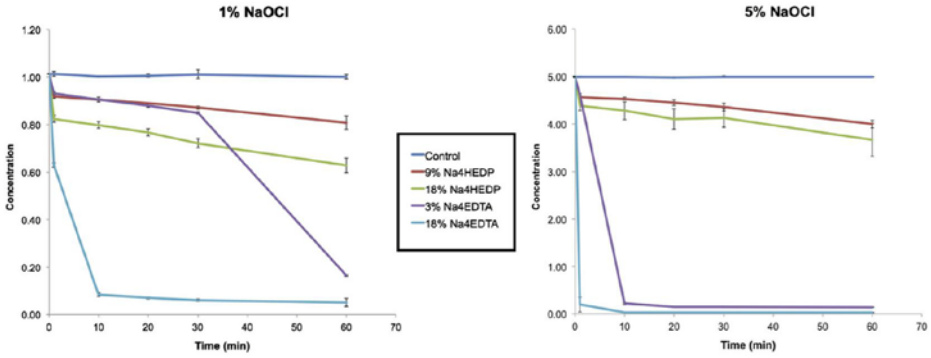

Figure 1. NaOCl concentrations in the 2 solutions used in this study over time as assessed by iodometric titration. Note that Na_4EDTA salt caused a faster decline in available chlorine in the 5% compared with the 1% NaOCl solution. This was not the case with the Na_4HEDP salt.

Discussion

The current study investigated the potential of adding particulate chelators to NaOCl solutions to generate an ad hoc all-in-one irrigant. NaOCl should maintain its pH and available hypochlorite (OCl^-) with an additional decalcifying effect during the approximate course of an endodontic treatment. This approach would circumvent the technical problems of storing and/or mixing solutions. The results showed that the tetrasodium salt of HEDP, but not necessarily that of EDTA, is suitable for this purpose, even if the EDTA concentration is kept low.

Although the current experiments were performed in a simulated clinical environment, this is a strictly chemical investigation, and no clinical measures were obtained. The chelator salts were mixed with sodium hypochlorite solutions at room temperature in polypropylene syringes. For the experiments on the effect of hypochlorite on the chelators, a 100-ppm Ca^{2+} solution was selected, because this amount of calcium corresponds to that removed from root canals during the irrigation with chelating solutions (13). It is clear from the available literature that all the effects of NaOCl solutions desired by the endodontist, ie, biofilm and necrotic tissue dissolution, are related to available chlorine and pH in that solution (21). HEDP in aqueous solution, when freshly mixed with a NaOCl solution, neither impedes the antimicrobial properties of the NaOCl (22) nor its ability to dissolve necrotic tissue (23). A recent study also showed that this may be the case with an alkaline solution of Na_4EDTA (15). The EDTA solution normally used by dentists has a pH between 7 and 8.5 and is prepared by adding sodium hydroxide (NaOH) to the disodium salt of EDTA in water. Such a solution strongly interferes with available chlorine (hypochlorite) in NaOCl solutions (24). Tartari et al (15) reported that Na_4EDTA, when mixed with NaOCl, continues to remove smear layer and has little effect on tissue dissolution in the short term. This is in seeming contrast to the current results. However, these authors mixed 5% NaOCl with solutions of 10% and 20% Na_4EDTA (resulting in 2.5% NaOCl solutions) and then assessed the smear layer (5 minutes) and soft tissue dissolution (15 minutes). The focus of the current study was different; we investigated the possibility to directly mix chelator salts with NaOCl solutions (resulting in higher available concentrations of chelator and hypochlorite), store these mixtures for different time intervals, and then assess their efficacy. The results reported here on the loss of available chlorine in NaOCl solutions are comparable to those reported by other authors; their solution initially containing 2.5% NaOCl and 10% Na_4EDTA lost 51% of the available chlorine in the first hour (15). The current results show that the more available chlorine and Na_4EDTA was in the system, the stronger was the chemical interaction between these 2 compounds (Tables 1 and 2). This can be explained as a result of kinetics, because the reaction rate is increased for higher concentrations of substrates. Consequently, the tetrasodium salt of EDTA does have some compatibility with NaOCl but only at low concentrations and in the short term. As reported earlier, this may be useful when 2 solutions are freshly mixed (15). For the application intended in this study and in the clinical situation, however, the tetrasodium salt of HEDP appears much more suitable than the EDTA counterpart.

The limited interference of the NaOCl solutions with the Ca^{2+} complexation of the chelators under investigation has also been reported in earlier studies assessing mixtures of the respective solutions (12, 13, 23). Although NaOCl is a strong oxidizing agent, it had little effect on calcium chelating ability. Comparing the mixture $HEDP/H_2O$ with HEDP/NaOCl, the calcium chelation was slightly reduced because of the oxidized chelator, which decomposes to acetic acid. Although there is also an oxidation of EDTA, the decomposition is rather slow (25) and can yield unknown byproducts and iminoacetic acid, which can still chelate Ca^{2+}. Hence, EDTA could possibly chelate Ca^{2+}, but NaOCl is also decomposed, and there is not enough available chlorine present if used in a combined solution (Tables 1 and 2).

Any time new materials or combinations (irrigants) are used in different combinations or with different techniques, there are many questions regarding biocompatibility and efficacy that need to be addressed. This article provides a first step to at least provide the feasibility for this irrigant combination. However, apart from the self-evident advantage for clinicians to use just one irrigant, it remains unclear whether the effective concentrations of chlorine and the capacity of a chelating agent maintained better when undiluted stable individual components are used and frequently refreshed or a single component is refreshed but constantly degrading during the hour of use. Future studies should also address the compatibility of Na_4HEDP with chlorhexidine, $Ca(OH)_2$, as well as etching and bonding agents. The effects of temperature and ultrasonic activation on the combined irrigant should also be assessed. Furthermore, dentin conditioning by using a NaOCl/HEDP combination should be scrutinized regarding its effects on pluripotent cells/regenerative endodontic procedures. Last but not least, clinical studies should be performed. Indeed, it is not entirely clear whether a decalcifying agent is truly necessary to improve the outcome of root canal treatment, although follow-up studies gave a hint in that direction, at least in the case of retreatments (26).

Conclusion

The compatibility and solubility of particulate Na_4HEDP with/in NaOCl solutions are such that these components can be mixed and used for up to 1 hour. In contrast, short-term compatibility of the Na_4EDTA salt with NaOCl solutions was considerably lower, decreasing at higher concentrations of either compound. Especially for Na_4HEDP but also for Na_4EDTA, NaOCl had little effect on calcium complexation. However, it needs to be cautioned that these statements are based entirely on a laboratory study model and not on actual clinical conditions.

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Dirk Mohn and Matthias Zehnder declare a potential conflict of interest. They are both shareholders of smartodont llc, which applied for a patent related to the basic idea of using particulate Na_4HEDP in endodontic irrigants and in combination with calcium hydroxide (EP16184212). The remaining authors deny any conflicts of interest related to this study.

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Corrigendum to 'WNT6 Promotes the Migration and Differentiation of Human Dental Pulp Cells Partly through c-Jun N-terminal Kinase Signaling Pathway' [J Endod 2014;40:943–948]. Ruimin Li, DDS, PhD, Chenglin Wang, DDS, PhD, Juan Tong, DDS, MD, Yingying Su, DDS, PhD, Yunfeng Lin, PhD, Xuedong Zhou, DDS, PhD, and Ling Ye, DDS, PhD. State Key Laboratory of Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, Sichuan, China.

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The authors would like to apologise for any inconvenience caused.

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Corrigendum to 'EZH2, a Potential Regulator of Dental Pulp Inflammation and Regeneration' [J Endod 2014;40:1132–1138]. Tianqian Hui, PhD, Peng A, PhD, Yuan Zhao, PhD, Chenglin Wang, PhD, Bo Gao, PhD, Ping Zhang, PhD, Jun Wang, PhD, Xuedong Zhou, PhD, and Ling Ye, DDS, PhD. State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Sichuan, China.

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